lifelong continuity of cell phenotype and cell fate determination. This founding assumption was based on the readily observable fact that cells stably maintain their phenotype over a lifespan, implying stable underlying biochemical epigenetic mechanisms. The principal molecular cornerstones of this presumed stability were DNA cytosine methylation and the long-lived histone subunits that comprise the octameric core of the chromatin particle. Thus, the basic assumption was that "irreversible" chemical reactions underlay cellular information storage. Work over the last decade has made clear that these early models of epigenetic stability are incorrect. Thus, it has been discovered that DNA cytosine methylation is chemically reversible in nondividing cells, as is histone lysine/arginine methylation, and most recently, breakthrough work that includes these new finding by Maze et al. indicates that dynamic regulation of the chromatin core particle can be added to this list (Zovkic et al., 2014).

It is clear that transient signals can trigger lifelong changes in cell function, and by definition, epigenetic mechanisms contribute to this form of cellular information storage. However, the prior model of epigenetic changes being permanent due to an underlying magic bullet of irreversible chemical reactions needs to be replaced by an understanding of cellular persistence being subserved by ongoing dynamic but bistable biochemical reactions (Nicolis and Prigogine, 1977). The costs of dynamic bistability are 2-fold—a requirement for ongoing energy input to defeat the second law of thermodynamics, and the possibility of accumulation of errors. In the case of cells, the cost of errors is phenomena such as aging, memory degradation, and oncogenesis. However, the evolutionary benefits of cellular changes being subserved by dynamic bistable reactions are manifold—allowing the acquisition of acquired change and such phenomena as stimulus-induced homeostatic regulation, cellular and neural plasticity, and organismal learning.

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Dissecting the Role of Smooth Muscle Cells versus Pericytes in Regulating Cerebral Blood Flow Using In Vivo Optical Imaging

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The brain regulates blood flow to match energy demand to nutrient supply. In this issue of *Neuron*, using in vivo optical imaging and optogenetics, Hill et al. (2015) report that arteriolar smooth muscle cells are key players in regulating cerebral blood flow in the healthy state and contribute to the "no reflow" phenomenon after ischemic stroke.

To meet the energy demands of the CNS, the vasculature modulates cerebral blood flow (CBF) through neurovascular coupling, a process by which blood vessels respond to regions of high neuronal activity by vasodilation and increased flow (Girouard and Iadecola, 2006). These changes in microcirculation are driven by release of neurotransmitters that promote

arteriole dilation to meet the metabolic needs of local neurons (Attwell et al., 2010). Multiple components of the neurovascular unit (NVU) have been implicated in regulation of neurovascular coupling; however, the contractile properties of mural cells surrounding the blood vessels ultimately play a fundamental role in controlling vessel diameter and altering CBF (Bell et al., 2010; Girouard and Iadecola, 2006). Among mural cells, pericytes are critical structural and functional components of the NVU that are required for CNS angiogenesis, as well as formation and maintenance of the blood-brain barrier (BBB) (Armulik et al., 2010; Daneman et al., 2010; Hellström et al., 2001). During embryonic development, pericytes are recruited to stabilize nascent vessels, an interaction consolidated by N-cadherin at the endothelial-pericyte interface. Microvessels of the CNS have the highest pericyte coverage of any organ, with approximately 30% coverage of the abluminal surface of the endothelium (Armulik et al., 2011). Mature pericytes share a basement membrane with endothelial cells and localize to terminal arterioles, capillaries, and postcapillary venules (Armulik et al., 2011; Díaz-Flores et al., 2009).

While pericytes have many important functions in the CNS vasculature, their involvement in the regulation of CBF remains controversial for several reasons (Armulik et al., 2011). First, the morphological and genetic markers of pericytes compared to smooth muscle cells (SMCs) are not absolute. In the CNS, pericytes are characterized by an elongated flattened shape, with multiple processes that run along capillaries. They express platelet-derived growth factor receptor-beta (PDGFRβ), NG2, alpha-smooth muscle actin (α-SMA), desmin, and RGS5; however, expression of these markers varies both developmentally and among tissues (Krueger and Bechmann, 2010). For example, quiescent pericytes that surround capillaries do not express α-SMA; nevertheless, α-SMA expression is rapidly upregulated in blood vessels associated with tumors (Morikawa et al., 2002). On the other hand, ultrastructural and immunohistochemical studies have found that a subset of CNS pericytes expresses contraction-related proteins including α-SMA and desmin (Liu et al., 2012). Moreover, pericytes are very plastic cells, and they behave differently in slice preparations compared to the intact brain. Pericytes can promote capillary constriction in ex vivo preparations of retinal and cerebellar slices, and they respond to neurotransmitters with contractile force (Peppiatt et al., 2006). Fernández-Klett et al. (2010) found that although pericytes can also dynamically alter capillary diameter in vivo with two-photon imaging, they are not responsible for increased CBF in response to neural activity (Fernández-Klett et al., 2010).

The "contractile" properties of pericytes have been suggested to contribute to the deterioration of CNS vascular reperfusion following ischemic stroke. Pericytes have been described to constrict capillaries within the first few hours after the onset of simulated retinal ischemia or cerebral ischemia following transient middle cerebral artery occlusion (t-MCAO) (Liu et al., 2012). A subset of pericytes, termed filamentous pericytes, have been attributed with the control of blood flow in response to neuronal activity; these cells may have a more detrimental role for stroke progression because they contribute to persistent constriction of capillaries that further lowers oxygen supply in tissue subjected to ischemic injury (Liu et al., 2012). The persistent constriction of capillaries due to their death has also been proposed to prevent tissue perfusion, leading to the "no reflow" phenomenon (Hall et al., 2014). A major confusion in this field has been a lack of clear molecular markers that distinguish whether these cells are a subset of SMCs that cover the pre-capillary arterioles or capillary pericytes. This has raised doubts as to whether pericytes are in fact the mural cell responsible for neurovascular coupling.

The study by Hill et al. (2015) addresses this important question: namely, which mural cell types— SMCs that cover arterioles or pericytes that cover capillaries— control neurovascular coupling and regulate CBF in the brain. The authors genetically labeled mural cells (SMCs and pericytes) by crossing either NG2::Cre or NG2::CreER transgenic mice with the mT/mG reporter strain. This approach allowed them to identify four distinct cell morphologies corresponding to mural cells that cover arteries, arterioles, capillaries, and post-capillary venules. Surprisingly, pre-capillary arteriolar SMCs and capillary pericytes have distinct morphologies; arteriolar SMCs display a more circumferential band-like morphology, whereas pericytes have thin processes that extend longitudinally along multiple capillary branches, consistent with previous studies (Armulik et al., 2011). Moreover, arteriolar SMCs, but not capillary pericytes, express α-SMA in both human and mouse brains, suggesting that pericytes lack contractile properties.

The authors performed a variety of in vivo two-photon optical imaging experiments to resolve which mural cells are responsible for either spontaneous vasomotion or vessel relaxation in response to neural activity. Through two-photon imaging in live animals, the authors found that spontaneous vasomotion occurs in arterioles covered with SMCs, consistent with their structural and molecular findings. The authors then generated an NG2-Cre::GCaMP3 transgenic mouse strain to correlate changes in Ca^{2+} signaling in mural cells with vasomotion through in vivo imaging in awake animals. They consistently observed that Ca^{2+} fluctuations in arteriolar SMCs, but not capillary pericytes, correlate with vasomotion. The authors then performed optogenetic activation of arteriolar SMCs or pericytes in a novel transgenic mouse strain (NG2cre:ChR2-YFP) and found that stimulation of arteriolar SMCs but not pericytes promoted changes in vessel diameter. Finally, the authors imaged vascular response to whisker stimulation in the somatosensory cortex in awake mice. Consistent with previous experiments, whisker stimulation induced vasodilatation only in areas of the vascular tree that contain SMCs, but not in capillaries. These data provide strong evidence against the prevailing dogma that pericytes regulate cerebral vascular flow (Hall et al., 2014; Peppiatt et al., 2006) and point out that arteriolar SMCs may be the key players in this fundamental process.

Since contractile properties of pericytes have been suggested to contribute to the exacerbation of stroke pathology, the authors examined whether arteriolar SMCs or pericytes contribute to the focal restriction of microvessels following cerebral ischemia, a phenomenon that prevents reestablishment of blood flow. They employed the t-MCAO model for ischemic stroke, coupled with continued

two-photon imaging of SMCs and pericytes prior, during and 4 hr after occlusion. Contrary to previous studies (Hall et al., 2014), focal restrictions occurred in terminal arterioles covered with SMCs, but not in capillaries covered with pericytes. Therefore, SMCs and not pericytes are the critical mural cells that prevent reflow leading to irreversible microvascular occlusion and exacerbation of disease.

Why has it been so difficult to dissect out which mural cells contribute to neurovascular coupling? It is possible that pericytes may upregulate contractile proteins in slice preparations and contribute to capillary constrictions (Hall et al., 2014; Peppiatt et al., 2006), but they do not possess these properties in vivo (Hill et al., 2015). Since previous in vivo imaging studies found that pericytes can dynamically alter capillary diameter (Fernández-Klett et al., 2010), the contractile mural cells could have been misidentified as pericytes and not arteriolar SMCs. Precapillary arteriolar SMCs and capillary pericytes share many molecular markers and structural similarities. The strict separation between arteriolar SMCs and pericytes by Hill et al. (2015) is primarily based on α-SMA expression and the association with caliber vessels that would suggest arteriolae. Nevertheless, these two cell types (arteriolar SMCs and pericytes) could be actually more similar to one another both from a developmental and molecular point of view.

In conclusion, the study by Hill et al. (2015) reveals a novel function for arteriolar and pre-capillary SMCs in regulation of CBF both in health and disease. Pericytes are critical for regulation of BBB function in capillaries (Armulik et al., 2010; Daneman et al., 2010), and therefore, their role may be more restricted to dysregulation of endothelial barrier function following ischemic stroke. CNS pericytes have a developmental origin distinct from SMCs (Armulik et al., 2011). Since pre-capillary SMCs and pericytes share many molecular markers except α-SMA, they may represent either two distinct cell types with different developmental origins or a single plastic cell type that can change its phenotype along the vascular tree. Identification of novel molecular markers that discriminate among distinct structural or functional classes of SMCs and pericytes in the cerebral vascular tree will be a valuable tool in the future to parse out the role of these distinct cell populations in the healthy brain and during various CNS pathologies.

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